(f) *Calculations.* (1) Calculate the micrograms of cefuroxime per milligram of sample as follows:

$$\text{Micrograms of cefuroxime per milligram} = \frac{R_u \times P_s \times 100}{R_s \times C_u \times (100 - m)}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter;

C_u=Milligrams of sample per milliliter of sample solution; and

m=Percent moisture content of the sample.

(2) Calculate the cefuroxime content of the vial as follows:

$$\text{Milligrams of cefuroxime per vial} = \frac{R_u \times P_s \times d}{R_s \times 1{,}000}$$

where:

R_u=Area of the cefuroxime peak in the chromatogram of the sample (at a retention time equal to that observed for the standard)/Area of internal standard peak;

R_s=Area of the cefuroxime peak in the chromatogram of the cefuroxime working standard/Area of internal standard peak;

P_s=Cefuroxime activity in the cefuroxime working standard solution in micrograms per milliliter; and

d= Dilution factor of the sample.

[48 FR 38460, Aug. 24, 1983; 48 FR 40704, Sept. 9, 1983]

§ 436.344 Thin layer chromatographic identity test for cefuroxime.

(a) *Equipment*—(1) *Chromatography tank.* Use a rectangular tank approximately 23×23×9 centimeters, with a glass solvent trough on the bottom and a tight-fitting cover. Line the inside walls of the tank with Whatman #3MM chromatographic paper or equivalent.

(2) *Plates.* Use 20 × 20 centimeter thin layer chromatography plates coated with Silica Gel F or equivalent to a thickness of 250 microns.

(b) *Developing solvent.* Mix chloroform, methanol, and formic acid in volumetric proportions of 90:16:4, respectively.

(c) *Preparation of the spotting solutions.* Dissolve approximately 200 milligrams each of the working standard and sample in 5 milliliters of a 50 percent aqueous acetone solution.

(d) *Procedure.* Pour the developing solvent into the glass trough at the bottom of the chromatography tank. Cover and seal the tank. Allow it to equilibrate for 1 hour. Prepare a plate as follows: On a line 2 centimeters from the base of the plate, and at intervals of 2 centimeters, spot 5 microliters each of the sample and working standard solutions. After all spots are thoroughly dry, place the plate directly into the glass trough of the chromatography tank. Cover and seal the tank tightly. Allow the solvent front to travel a minimum of 15 centimeters from the starting line. Remove the plate from the tank and allow it to air dry. Observe under ultraviolet light (254 nanometers).

(e) *Evaluation.* Measure the distance the solvent front traveled from the starting line and the distance the spots are from the starting line. Calculate the R_f value by dividing the latter by the former. The sample and standard should have spots of corresponding R_f values.

[48 FR 38461, Aug. 24, 1983]

§ 436.345 High-pressure liquid chromatographic assay for ceftizoxime.

(a) *Equipment.* A suitable high-pressure liquid chromatograph equipped with:

(1) A low dead volume cell 8 to 20 microliters;

(2) A light path length of 1 centimeter;

(3) A suitable ultraviolet detection system operating at a wavelength of 254 nanometers;

(4) A suitable recorder of at least 25.4 centimeter deflection;

(5) A suitable integrator; and

(6) A 30-centimeter column having an inside diameter of 4.0 millimeters and packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles, 5 to 10 micrometers in diameter, USP XX.

(b) *Reagents*—(1) *pH 3.6 buffer solution.* Transfer 2.31 grams of sodium phosphate diabasic dodecahydrate and 1.42 grams of citric acid monohydrate to a